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THE RELATION BETWEEN THE CONCENTRATION OF
ADRENALINE AND ITS ACTION

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Thesis presented for the Degree of M.D.,
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October 1927.



The Relation between the Concentration of Adrenaline and its Action.

It is a remarkable fact upon which several authors have commented that, despite the great mass of research which has been undertaken into the manner in which drugs produce their effects upon the animal body, very few attempts have been made to determine the influence of the dosage of the drug upon its effects. This is the more surprising when it is borne in mind that an accurate understanding of the relation between the dosage and the effect of a drug is essential for the scientific administration of remedies. It is true that spasmodic attempts were made from time to time to repair this gap in the structure of pharmacological science, but these attempts lacked the accurate observation of simple reactions between the drug and the tissues which is the fundamental requisite in this branch of study, and were based too largely on speculative assumptions to constitute any advance. It is only within the last three or four years that the subject has been investigated along strictly scientific lines.

Studies in the relation between the concentration and the action of a drug fall into two large groups. In the one group, the action is an irreversible/

irreversible one; the cells are destroyed by the action of the drug. The disinfectants are the chief members of this group. The haemolysins also belong to this group and have been very thoroughly studied by several workers. When the action of such substances is examined it is found that the concentration-action curves have a characteristic S-shaped form. (By "concentration-action" curve is meant the curve which is obtained when the action expressed as the percentage of the maximum possible action is plotted against the corresponding concentration). The shape of these curves is explained as follows. It is assumed that the cells offer a definite resistance to the action of any agent which is liable to destroy them. But the resistances offered by individual cells vary considerably in degree and the sigmoid form of the curves is in fact due to these resistances being distributed among the cells in accordance with the law of probability.

In the other group are placed all those drugs whose action is reversible; that is the drug produces a stimulation or a depression of the special function of the tissue and from this stimulation or depression the tissue recovers completely when the drug is removed. Most of the drugs used in therapeutics belong to this group and among them is adrenaline. It might be anticipated that the concentration-action curves in this would have a different form from the frequency curves of the previous/

previous group, since the question of resistance to destruction by a toxic agent does not arise. This anticipation is realised by the work of Clark⁽¹⁾ and by the experiments which will be described later in this paper. Two independent investigators, however, claim to have obtained S-shaped concentration-action curves from adrenaline, a drug whose action is clearly reversible. These claims fall to be discussed in detail in a later section, when reasons will be brought forward for rejecting them as mistaken. Meantime it may be taken that the two groups of drugs exist and have a fundamentally different mode of action. As adrenaline is a member of the second group, the remainder of the discussion in this section will be confined to this group.

The next question to be considered is how drugs with reversible actions do produce their effects upon the cells of a tissue. The work of Straub⁽²⁾ was the first attempt to throw light upon this matter. Straub examined the actions of a number of drugs on the hearts of the frog and of *Aplysia*. He found that in the cases of veratrin, morphine, and strychnine, the maximum action coincided with the maximum content of the drug in the heart muscle cells themselves; whereas in the cases of muscarine and atropine a maximum action was produced although the heart muscle cells themselves contained little or no /

no drug; the surrounding fluid, however, had a high percentage of the drug. Thus he subdivided this group of drugs with reversible reactions into two sub-groups:

(a) Concentration poisons, of the veratrin type, in which the action depends on the concentration of the drug attained in the cells:

(b) Potential poisons, of the muscarine type. Straub applied the term "potential poison" to muscarine since the action appeared to depend on the difference in concentration of the poison within the cell and in the fluid surrounding the cell. He considered that pilocarpine and adrenaline also belonged to this group.

The potential poisons have attracted much more attention from investigators than the concentration poisons and as adrenaline is believed to be a member of the group, it is necessary to discuss them further. Straub's theory of their action was that as the drug entered the cell it produced a change at the surface which was responsible for the effect produced; this surface change was dependent on the actual transit of molecules through the cell membrane. Straub thought that the entry of the drug into the cell actually antagonised the action of the drug, the concentrations outside and within the cell becoming the same. This view has been challenged. It implies that when a solution of one of these potential§/

potential poisons is brought into contact with a tissue, the tissue undergoes a change of state from which it later recovers even although it is still bathed in the solution which produced the original change. But while such effects have been observed they are not invariable. Thus Clark ⁽¹⁾ points out that no recovery occurs when the frog's heart is exposed for long periods to atropine solutions, and the same is true of the action of nicotine on the rectus abdominis of the frog; moreover, even in the case of those drugs which do show the recovery effect, it is found to be inconstant. The action of these drugs therefore cannot be ascribed merely to the difference in concentration of the poisons outside and inside the cell; it is not due to a surface change which is dependent on the entry of the drug into the cell.

Recent work has, however, confirmed Straub's belief that these drugs act upon the surface of the cell. Thus Clark found that the action produced by acetyl choline on the frog's heart and the amount of acetyl choline taken up by the heart cells bore no relation to one another. Cook ⁽³⁾ in a study of the antagonism of acetyl choline by methylene blue found that heart cells adsorb methylene blue slowly, this adsorptive action being irreversible, but that the action of methylene blue in antagonising acetyl choline was rapidly produced and as rapidly removed by washing out the dye. He adds that a heart/

heart can regain its full sensitivity to acetyl choline although it is deeply stained by methylene blue. These experiments show very clearly that these drugs produce their effects by causing some change at the surface of the cell, and that this process is quite independent of the entry of the drug into the cell.

What is, then, the nature of this change which occurs at the surface of the cell? One possibility is that it may consist in the formation of a continuous layer of molecules of the drug over the surface of the cell. This is negatived, however, in the case of acetyl choline at any rate, by Clark's finding that the area of the cell surface is about two hundred times greater than the area occupied by the number of molecules required to produce an action on the cell. Another possibility is that the drug may undergo some sort of reversible combination with specialised receptors in the cell. This idea has been adopted by many workers and in particular unimolecular formulae have been applied to explain the relation between the concentration and the action of drugs. Arrhenius attempted to explain even some irreversible reactions, viz. haemolyses, in this way, but this suggestion is now discredited. In the case of reversible reactions however the case is different. For, turning again to Clark's work upon acetyl choline we find that acetyl/

acetyl choline acts upon the heart over a ten-thousand fold range of concentration in accordance

with the formula $Kx = \frac{y}{100 - y}$

where x = molar concentration of the drug

y = action expressed as percent. of maximal
action

K = constant

This at once suggests that the drug acts by combining with some receptor in the cell according to a unimolecular reaction. The number of receptors in the tissue must be assumed to be limited while the molecules of drug are in great excess. The success which attended the application of the formula mentioned to the actual observations suggested the need for an investigation to determine how far the actions of other members of the potential poison group could be explained in the same manner. The present paper describes a study of the action of adrenaline on the plain muscle coats of the arteries, from this point of view. The action of adrenaline on the blood pressure is afterwards discussed, after the fundamental relation between the concentration and the action has been established. The later discussion is concerned mainly with the various modifications introduced into the fundamental relation by the conditions of the circulation, physical or physiological.

Action of Adrenaline on Arterial Strips.

A series of experiments was performed on strips taken from the carotid arteries of sheep. The arteries were obtained from the slaughter-house. They were excised immediately on the death of the animal and were transferred at once to a bottle of ice-cold Ringer's solution in which they were stored until required for experiment. Arteries can be kept in this way for twenty-four hours at least without losing their sensitivity to adrenaline; after this period they may respond in regular fashion but in many cases they may contract on application of the first dose of adrenaline and then cease to respond; or they may develop spontaneous contractions.

Strips were prepared from the excised arteries in the following manner: A portion of the artery about 2 cms. long was stripped of adventitia and from this portion a ring 5 mm. long was cut out. The ring was then slit longitudinally so that a strip was obtained. Each end of the strip was now transfixated by a fine small silver pin passing through its corners. The distance between the two pins was now measured - it usually amounted ^{to} about 7-8 mm. The pins afforded the means of attachment of the strip to the recording apparatus. The lower pin was fastened to a hook formed on the end of the glass tube by which aeration of the solution bathing the preparation was obtained. The upper pin was connected/

connected by a thread to an isometric lever. This lever consisted of a straw attached to a thin strip of spring steel; when a weight of 1 gram was hung on the strip of steel, the writing point on the straw showed an excursion of about 1 cm.

The bath in which the arterial strip was suspended contained about 32 cc. of fluid, but to ensure accuracy the actual amount of outflow was measured when the solution causing a contraction was washed out. The bath was placed in a copper vessel containing water maintained at a constant temperature of 37° C. by means of an adjustable electric lamp. Special precautions were taken to ensure that the Ringer's solution entering the bath from the reservoir should also have a temperature of 37° C.; it had to pass through a small reservoir and through a spiral of glass-tubing, both of which were placed in the warm water jacket surrounding the bath. Diffusion backwards of adrenaline solutions from the bath through this system of tubing was prevented by placing the clip regulating the inflow of solution into the bath also in the water jacket close to the bath. The solution employed had the following composition:

NaCl	0.9%
KCl	0.04%
CaCl ₂	0.02%
NaHCO ₃	0.02%
P _H	8

A 1:1000 solution of adrenaline was made up as required from a synthetic preparation, a few drops of dilute hydrochloric acid being added to dissolve the adrenaline. In none of the experiments was it found that the amount of adrenaline solution added to the bath was sufficient to alter appreciably the P_H of the Ringer's solution in the bath. During an experiment dilutions of 1:10,000; 1:100,000; 1:1,000,000 were made up as required and quantities of from 0.05 to 1 cc. were added direct to the solution in the bath. The constant aeration of the fluid in the bath ensured rapid admixture of the solution of the drug with the Ringer's solution in the bath. The concentration of adrenaline attained was determined at the end of each trial by measuring the outflow.

Before making any estimations of the effect of adrenaline on the strip, the strip was lengthened by slowly raising the stand to which the isometric lever was attached. This procedure was suggested (4) by the work of Winton on the influence of length on the response of plain muscle to various stimuli. Winton found that the isometric tension developed by the retractor penis in response to electrical or chemical stimuli increases with the length up to a maximum; furtherlengthening of the muscle then diminishes the response. A few trial experiments showed that in the case of the arterial strips, the maximum responses were obtained when the initial length/

length had been doubled. The strips were accordingly stretched to this extent. This stretching of itself raised the tension very considerably, but then a gradual fall of tension occurred until a stable value was obtained. This base line was attained within an hour of the initial stretching and remained constant throughout the experiment.

Results were recorded on a slow moving drum, the paper being very lightly smoked. Constant attention was given to the writing-point to prevent friction on the drum producing errors.

The experiments were performed in the following manner. A fairly large initial dose was given an hour after the strip had been lengthened; this produced a rise of tension which differed considerably from the responses subsequently obtained from the same dose; sometimes the initial response was larger, in other cases it was smaller than the others. It was accordingly discarded. The subsequent contractions were quite regular. Care was taken to alternate large and small doses so as to eliminate the effects of any progressive change in the sensitivity of the strips. As it was found that large doses, producing an action 80 per cent. of the maximal or more, tended to impair the subsequent responses, they were given at the conclusion of the experiment.

The/

The strips began to contract at once as soon as the adrenaline was added to the solution. The records show that the tension rises sharply at first but that this rise becomes more and more gradual; with large doses the tension takes about twenty minutes to reach its maximum. The slow development of the action is in marked contrast to the rapidity which Clark and Cook found to characterise the actions of acetyl choline and methylene blue; but this is probably due to the fact that these drugs were studied on the heart, which in general responds to stimuli much more rapidly than plain muscle. As soon as the tension began to fall, the solution was removed and fresh Ringer was introduced; the strips, however, took a long time to relax, especially with the higher concentrations of adrenaline when more than an hour was required. This limited the number of estimations which could be made on one strip to six or seven, as after seven or eight hours in the bath the sensitivity of the strip becomes impaired. The attempt was made to hasten relaxation by the use of nitrites as recommended by Janeway, Richardson and Park⁽⁵⁾. 0.5 cc. of 1:10,000 sodium nitrite solution in distilled water was added to the bath but while this certainly produced relaxation some irreversible change occurred in the muscle, for even after repeated washing out of the nitrite solution the preparation failed altogether in many cases to respond/

respond to adrenaline.

Spontaneous movements of vessels have long been known to occur. Several observers have published records of such movements in excised arterial rings and it is believed that adrenaline is especially liable to induce such phenomena. In no case in the present series of experiments, however, were spontaneous movements observed provided that the artery had been kept, in ice cold Ringer's solution, for less than twenty-four hours prior to its use in an experiment. When the arteries had been kept for a longer period before use, in some cases spontaneous movements did occur, usually after one or two doses of adrenaline. These movements manifested themselves as a very rapid rise of tension, reaching a maximum within a few seconds of the onset of movement; relaxation from this condition was very slow and was sometimes interrupted by small secondary rises of tension. The frequency of such spontaneous contractions was usually very slow; the big waves occurred about once every seven or eight minutes. The rhythm was quite irregular. They passed off completely after four or five contractions at most. Such experiments were of course discarded.

The effect of temperature on the response of the strip to adrenaline was examined in only one experiment. It was found that no appreciable difference /

difference existed between the responses at different temperatures; for example at 32° C. the strip responded as strongly to a particular contraction of adrenaline as at 37° C. This is in accord with Clark's results for the action of acetyl choline upon the frog's heart. If the explanation which has been given for the action of acetyl choline is true also of adrenaline, we should not expect temperature differences to modify the responses to a given concentration of adrenaline, since temperature changes alone should not alter the equilibrium constant of a reversible reaction system.

The tensions developed by different strips varied widely. In some cases the maximum tension developed amounted to as much as 10 grams, in others it was less than 3 grams. It is of interest to note here that Barbour⁽⁶⁾ found that the responses of the distal end of the carotid were five times more powerful than those of the proximal end. This difference in the absolute amounts of tension developed by different strips in response to the same concentrations of adrenaline does not affect the results given since the action produced on a strip has been expressed not in terms of the absolute tension developed but as a percentage of the maximal action.

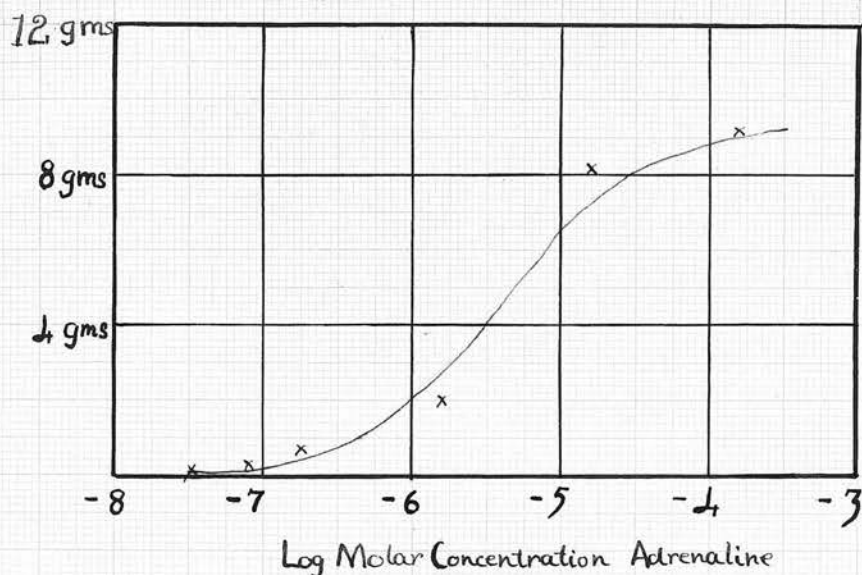
The arterial strips responded to adrenaline over/

over a five thousand-fold range of concentration, namely from 4×10^{-8} to 2×10^{-4} molar. Some difficulty was experienced in determining what should be taken as maximal action in each case, since, as has already been explained, the response becomes impaired after high concentrations of adrenaline. On the other hand the response to low concentrations could be determined with accuracy. The values so obtained were plotted against concentration and curves were drawn through these points for each experiment. Owing to the wide range of concentration it was necessary to employ a logarithmic scale for this: the effect of this is to alter the form of the curves for when ordinary numbers are used, these curves are of hyperbolic form. The logarithmic scale gives them a sigmoid form. A specimen curve is shown in Fig. 1. The crosses represent the tension actually developed at the corresponding concentration of adrenaline; the curve has been drawn according to the formula

$$Kx = \frac{y}{100 - y} \quad (\text{where } K = 252,000)$$

Fig. 1 /

Fig. 1.



Comparison of the curves -

It is not possible to compare the curves directly since the absolute tensions developed are so diverse, but it was found that all the strips had a similar sensitivity. This point is brought out in Table I. Table I was compiled from the curves drawn for each experiment through the points plotted according to the data supplied by the experiment. From the contours of these curves the tension which should be maximal for each particular strip was determined. The concentrations which produced 20 per cent., 50 per cent, and 80 per cent. of these maximal tensions were then read off from the curves.

Table I /

Table I.

Action produced (expressed as percentage of maximal action) -		20	50	80
Molar Concentration (logarithm)				
Expt.	1	$\bar{6}.2$	$\bar{6}.7$	$\bar{5}.2$
"	2	$\bar{7}.7$	$\bar{6}.7$	$\bar{5}.6$
"	3	$\bar{7}.9$	$\bar{6}.5$	$\bar{5}$
"	4		$\bar{6}.5$	$\bar{5}$
"	5	$\bar{6}.1$	$\bar{6}.6$	$\bar{5}.1$
"	6	$\bar{7}.9$	$\bar{6}.6$	$\bar{5}.2$
"	7	$\bar{6}$	$\bar{6}.5$	$\bar{6}.8$

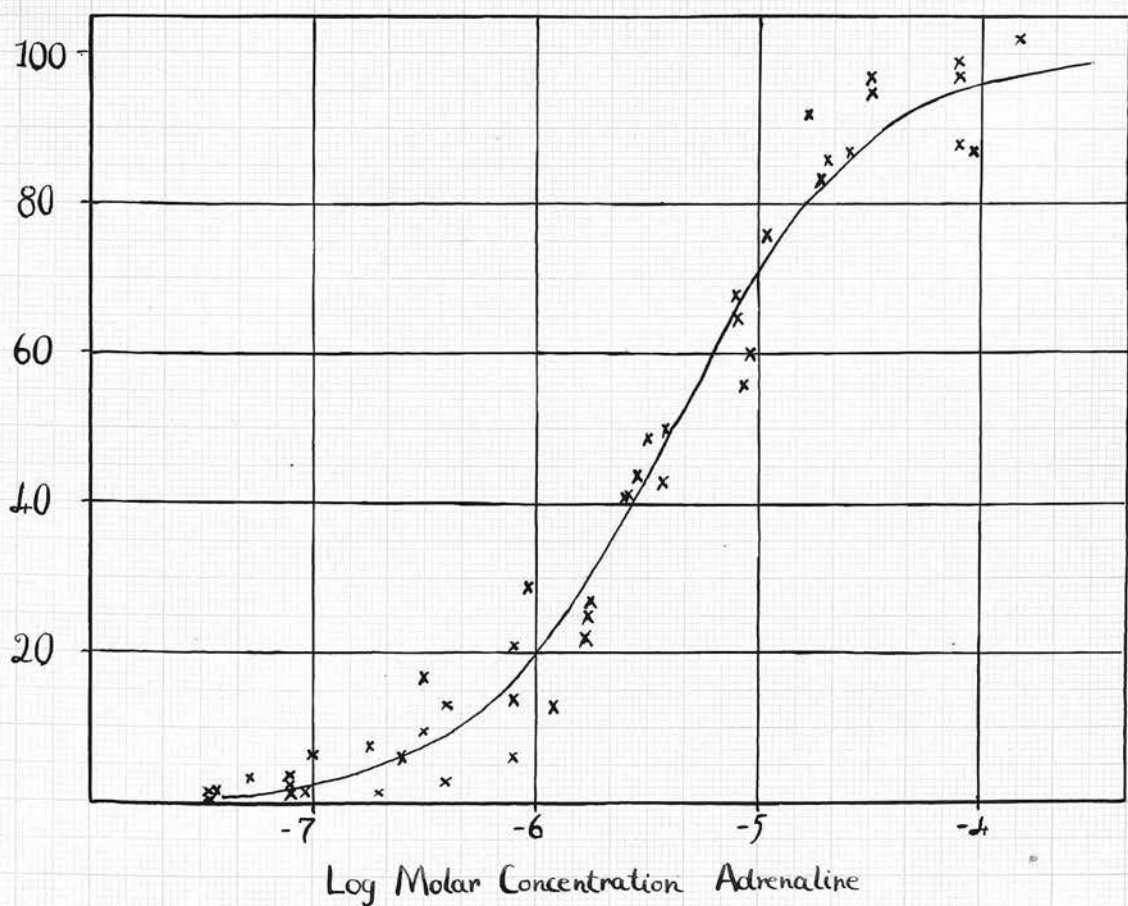
The table shows that in all cases concentrations in the neighbourhood of 4×10^{-6} molar (or $\bar{6}.6$ in logarithmic notation) produce actions 50 per cent. of the maximal. The tensions corresponding to the molar concentration 4×10^{-6} were now noted from the curve of each experiment. The figures so obtained were believed to be 50 per cent. of the maximal action and therefore the tensions produced by the concentrations which were actually used in each experiment could be converted to percentages of the maximal action. The figures obtained in this/

this way have been plotted in Fig. 2 where they are indicated by crosses; as in Fig. 1 the continuous line is the equation

$$Kx = \frac{y}{100 - y} \quad (\text{where } K = 252,000)$$

The figure shows a satisfactory agreement between the observed and the calculated values.

Fig. 2.



An attempt was made to determine the maximal action more directly. A chamber for electrical stimulation was made according to Winton's (4) recommendations. It was hoped in this way to produce a maximal contraction by a strong faradic stimulus, but these experiments were failures, the contraction produced being always weaker than when full doses of adrenaline were given.

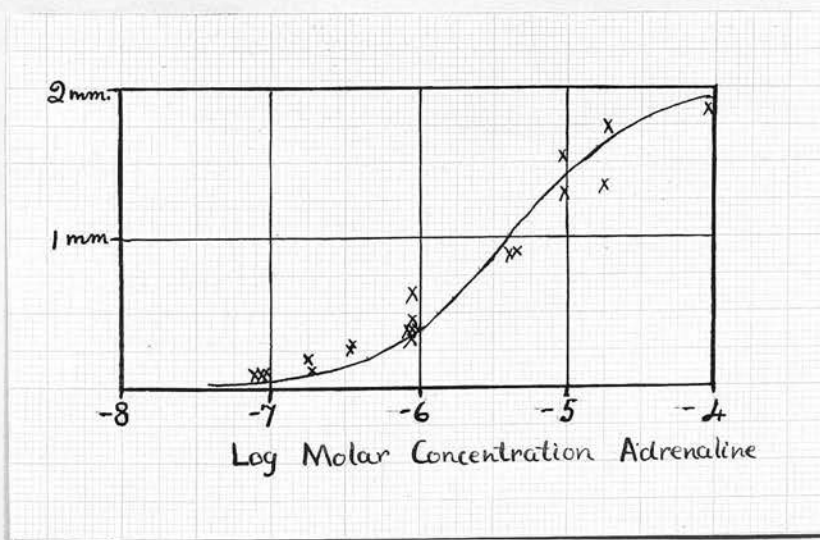
Isotonic experiments.

In addition to the experiments already described in which changes in tension were observed while the length of the strip remained constant, a few experiments were performed in which the changes in length were observed, the tension remaining constant. For this purpose an isotonic lever of the ordinary type was employed. The thread connected to the upper end of the strip was attached to the short arm of the lever so as to give a magnification of ten times. The strip itself was prepared in the manner already described. In this case stretching of the artery was obtained by hanging a weight on the long arm of the lever so as to produce a 10 gram tension for twenty minutes; this lengthened the strip by 4-5 mm. but on removal of the weight the strip shortened to a certain extent although it never returned to its initial length. As a rule, the permanent lengthening produced amounted to 1.5 to 2 mm. /

mm. As a whole this series of experiments was less successful than the preceding series of isometric experiments; imperfect relaxation after an adrenaline solution had been washed out of the bath was more common, and strips which gave erratic responses were met with. These difficulties are probably due to the difficulty in securing adequate preliminary stretching of the strip in this class of experiment.

The results of two experiments are shown in Fig. 3. It happened in both cases that maximal action was a contraction of 2 mm. and both strips had a similar sensitivity. They have therefore been graphed together. In Fig. 3, the crosses represent the number of mm. of shortening produced, while the continuous line is the same equation as the continuous lines in Figs. 1 and 2. The agreement between the observed and calculated results is quite satisfactory.

Fig. 3.



Reference has already been made to two independent workers who have worked along lines similar to those of the experiments which have just been described and who have arrived at conclusions very different from those reached here. It is necessary now to consider their findings in some detail.

(7)

Shackell studied the isotonic responses to adrenaline of arterial rings taken from the carotid of the sheep. The range of concentration which he examined, however, was only from 200×10^{-8} to 4000×10^{-8} molar whereas in my experiments graded responses were obtained even in the isotonic series over a range of concentration from 10×10^{-8} to $10,000 \times 10^{-8}$ molar, while the full range is seen from Fig. 2 to be from 3×10^{-8} to $20,000 \times 10^{-8}$ molar. Shackell obtained S-shaped concentration action curves over the range of concentration mentioned. To explain these curves he has advanced a theory based on the assumption that the "All or None" law which has been securely established for cardiac muscle and peripheral nerves applies to plain muscle also; the individual fibre contracts maximally or not at all when brought into contact with any solution of adrenaline. The curves therefore showed that the susceptibilities of the fibres to adrenaline are distributed in accordance with/

with the Law of Probability. Objection may be taken to this hypothesis both on theoretical and on experimental grounds. In the first place there is no evidence that the All or None Law does indeed apply to plain muscle. In the second place the curves which I have obtained over a much wider range of concentration are not sigmoid; they are hyperbolic and cannot be explained in the manner suggested by Shackell. S-shaped curves were obtained by Shackell's co-workers from the actions of strychnine and of chloral hydrate on the frog. But in these cases what was measured was the activity of a series of dilutions of the drug in causing the death of the animal, the time being chosen as the index of the activity. They were in fact studying irreversible reactions so that their results need not be further discussed here.

(8)

Gaddum studied the isotonic response of the rabbit's uterus to adrenaline. He obtained a series of S-shaped concentration-action curves when the concentration was plotted on a logarithmic scale; these curves are very similar to the curves which have already been figured. Of these he writes as follows: "The form of the curve is that which would be obtained on the assumption that the drugs acted on a number of units whose susceptibility is distributed about a mean in accordance with a probability curve; it/

it is the curve of the integral of the normal distribution".

This astonishing statement completely ignores the effect which the plotting of the concentration on a logarithmic scale has upon the shape of the concentration action curve. When the concentration is plotted in natural numbers the curve is at once seen to be a hyperbola which of course cannot be interpreted as Gaddum supposes. Clark ⁽¹⁾ has pointed out that Gaddum's results can be fitted closely by the equation $Kx = \frac{y}{100 - y}$.

From the evidence which has been brought forward in this section regarding the action of adrenaline on isolated arterial strips we may conclude that arterial muscle does respond to adrenaline in accordance with the formula $Kx = \frac{y}{100 - y}$

where x = molar concentration

y = action expressed as per cent. of maximal action

K = constant.

The significance of the formula may be stated as follows. The cells of the tissue have a limited number of receptors which may combine with molecules of adrenaline. When a solution with a large excess of adrenaline molecules bathes the tissue combination does occur. This reaction between these receptors and adrenaline is a reversible one; therefore at a given concentration of adrenaline the reaction attains/

attains a definite equilibrium so that at any instant the same proportion of the receptors is combined with adrenaline molecules although the particular receptors which are combined change every instant. At a higher concentration of adrenaline the point of equilibrium is shifted so that a higher proportion of the receptors becomes combined with adrenaline molecules. Associated with this combination of receptors and adrenaline molecules and depending directly on the proportion of receptors which become so combined, a definite degree of contraction of the cells occurs. The y of the formula has been said to express the action, i.e. the contraction of the cell, as a percentage of the maximal action; it stands also for the proportion of combined receptors. But while we see that the relation between the proportion of combined receptors and the occurrence of contraction in the cell is very intimate, we are unaware of its precise nature. Further the "receptors" which we have spoken of are of course hypothetical and we have no information as to their nature at all.

Action of adrenaline on the aorta of the frog.

In the preceding sections the fundamental reaction between adrenaline and the plain muscle cells of the arteries has been discussed. A formula has been stated which fits the concentration-action curves/

curves over the whole range of concentration in which the reaction is obtained - the same formula which Clark has applied to the reaction between acetyl choline and the cells of the frog's heart. The meaning to be attached to the formula has also been explained. Isolated tissues had to be used for this investigation since conditions in the body introduce numerous complicating factors into the relation between the concentration and the action of the drug. But, having determined the fundamental reaction between tissues and drug we can now proceed to a discussion of the action of the drug in the intact animal, namely, the rise of blood pressure which it produces. To do so, it is necessary to study the effect of introducing each of these complicating factors singly into the reaction. This is a task of very considerable magnitude. In the present paper only one has been studied, the effect which different concentrations of adrenaline in the perfusing fluid produce upon the outflow from an artery of small calibre.

In these experiments, a preparation which could be used at room temperature had obviously many advantages over one which had to be kept at a constant temperature of 37° C. The only mammalian preparation which fulfils this requirement is the rabbit's ear. A few experiments were made with this preparation. A cannula was inserted into the central artery at the/

the base of the ear. The tip of the ear was removed by a transverse cut about 1 inch proximal to its extremity; this cut of course sectioned the central artery of the ear. The attempt was made to occlude some small branches of the central artery which were necessarily divided at the same time by applying clamps to either side of the point from which the main flow came. The experiments were unsatisfactory; the length of the artery was too short and the difficulty of clamping all the branches while leaving the main artery intact, laid the experiments open to serious objection. This preparation was therefore abandoned as impracticable.

As an alternative the frog suggested itself for study. The frog is of course poikilothermic so that the perfusing fluid can be kept at any suitable temperature. The aorta of *Rana esculenta* was accordingly selected as the subject of this series of experiments. The preparation was made in the following manner. A cannula was tied into the left aortic arch by a ligature which occluded the left subclavian artery; this cannula was directed distally from the heart. The coeliac artery and the abdominal aorta were tied off as close to the junction of the two aortic arches as possible. The right aortic arch was divided at the origin of the right subclavian artery, The head, the fore-limbs, and/

and the heart were now cut away, for it was found in some of the earlier experiments that their presence tended to interfere with the free exit of fluid from the preparation by causing "pocketing" of the fluid if a slight movement of the preparation occurred. The preparation was then pinned securely on to a piece of paraffined cork tilted so that the divided end of the right aortic arch was the most dependent part of the preparation.

The fluid used for perfusion had the same composition as that recommended by Clark ⁽¹⁾, namely;

NaCl	0.65 per cent.
KCl	0.024 "
CaCl ₂	0.042 "
NaHCO ₃	0.05 "
P _H	8

Two Mariotte flasks were used as reservoirs to ensure a constant pressure on the vessel. One of them contained the solution alone; the other contained solution with a definite concentration of adrenaline present. The addition of adrenaline was made to a measured amount of the solution immediately before it was required for use, a precaution rendered necessary by the rapidity with which the higher dilutions of adrenaline became inert in alkaline solution. By the use of a three-way tap, the perfusing fluid could be admitted to the preparation from/

from either of the Mariotte flasks as desired. The connection between the tap and the cannula in the left aortic arch was a very short piece of rubber tubing. This rubber connection was kept short so that when the change from one reservoir to the other was made the new solution reached the preparation as soon as possible with a minimum of mixture with the previous solution.

The preparation being arranged as described, fluid thus entered the cannula in the left aortic arch from one or other of the Mariotte flasks. It passed along the left aortic arch, then along the right aortic arch, and escaped from the divided end of the right aortic arch on to the paraffined cork from the pointed dependent end of which it fell into a receiver. The average length of the vessel was about 3.5 cm.; its calibre was approximately uniform throughout. A constant pressure of 40 cm. of water was maintained on the preparation in every case. The rate of flow was estimated by counting the number of drops falling from the cork in half minute periods; an average rate of flow was about 25 drops per minute.

Reference has already been made to the occurrence of spontaneous rhythmic contractions in the case of isolated arterial strips. The same phenomenon has been observed by McDowall⁽⁹⁾ in the case/

case of perfusion experiments. McDowall perfused the pulmonary circulation of a series of rabbits and cats with a modified Ringer Locke solution (containing no bicarbonate) and found that waves of contraction and relaxation occurred at more or less regular intervals. These waves were stimulated by several agencies among which was adrenaline; their usual rate was about 3 per 2 minutes. They caused complete occlusion of the pulmonary circulation. McDowall states that similar waves are to be found when portions of the systemic circulation are perfused. It seems likely that the composition of the perfusing fluid has something to do with the production of this phenomenon. Certainly in no case were variations of the flow observed in the present series of experiments suggestive of spontaneous contractions on the part of the vessel walls, such as have been described by McDowall. In the course of a long experiment however the rates of perfusion, after the effects of each adrenaline perfusion had passed off, varied to a certain extent. For example, in the experiment recorded in Table II, which lasted fourteen hours, the rates of perfusion after recovery from adrenaline varied from 19 to 31 drops per minute. (The criterion of complete recovery from the adrenaline perfusion was taken as persistence of the outflow at the same rate for ten minutes at least). But the changes of tonus in the vessel which/

which must have caused this variation were so slow that they could not have affected the responses to the adrenaline solutions more than to the extent of one drop per minute if indeed they affected them to this extent which is unlikely.

Each experiment was begun by allowing the perfusion fluid to wash through the vessel for at least an hour before any adrenaline was allowed to pass through. During this time the flow was quite irregular due doubtless to the presence of serum and other constituents of the blood lying in the lumen. Thereafter a regular outflow developed and adrenaline perfusions could be begun. The first response to adrenaline, and sometimes the second also, were found to be irregular giving a response smaller than the same concentration later on; they were therefore discarded. As in the earlier experiments on the arterial strips high and low concentrations were alternated in irregular order to eliminate changes in sensitivity of the preparation, and each experiment was concluded by a very high concentration intended to produce a maximal action. The same tardy development of the full effect of adrenaline was seen here as in the experiments on the arterial strips, and recovery, though on the whole somewhat more rapid than in the strip experiments, was also slow. Difficulty was experienced in determining what constituted the maximal action, apparently from the very/

very slow rate of perfusion not permitting the maximal point to be observed. In no case was a rate of flow less than three drops per minute observed. It seems possible that at this rate of flow the amount of solution within the vessel must have been so small that the adrenaline became rapidly inert while the slowness of perfusion prevented fresh solution from reaching the vessel quickly enough to counteract this effect.

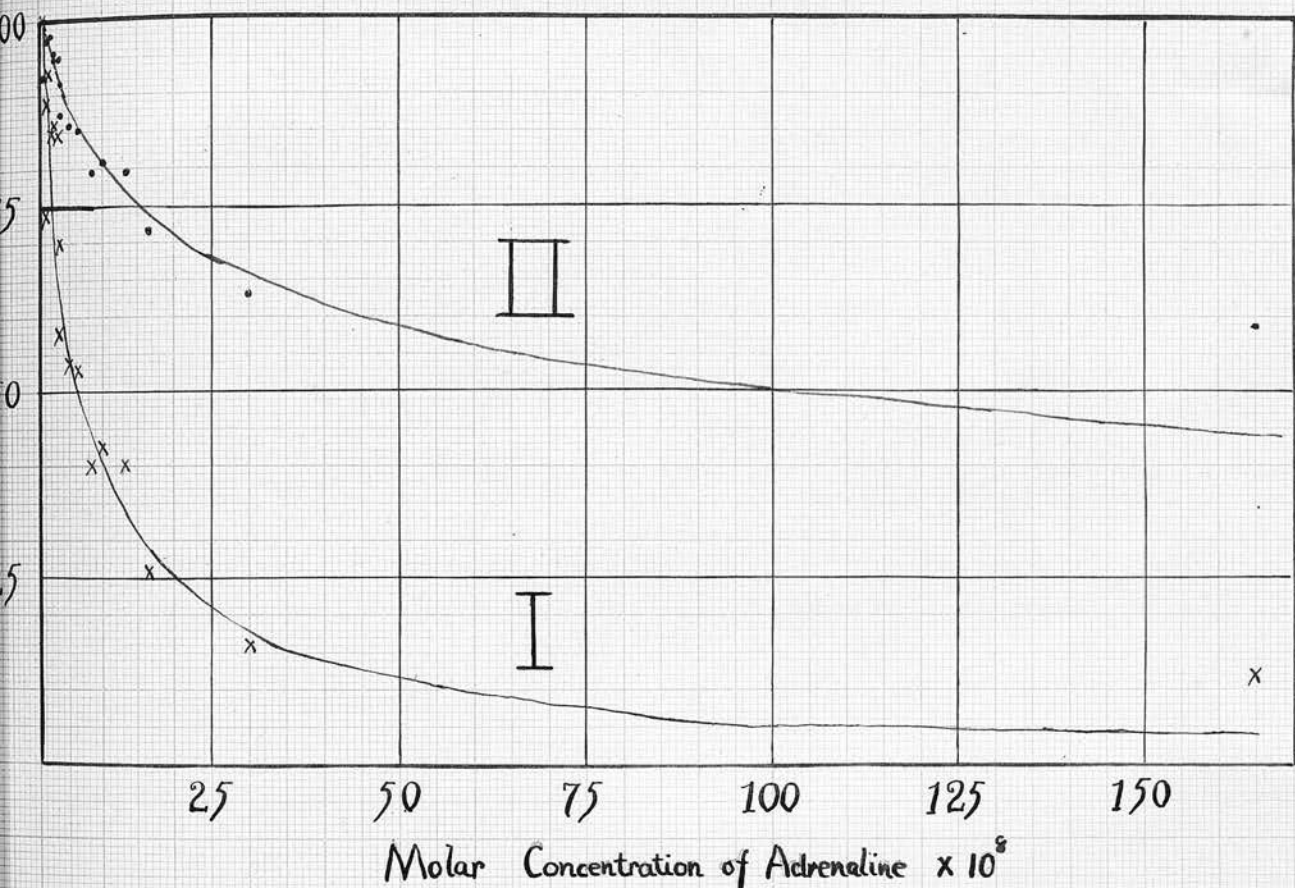
Results.

In determining the effect of the adrenaline solutions the ratio of the amount of diminution of outflow to the normal outflow, that is to the outflow immediately before the particular adrenaline perfusion was begun, was taken as the index rather than the number of drops by which the outflow was diminished, since the results given by the former method were more nearly constant. A specimen concentration-action curve is given in Fig. 4 (Curve 1). The crosses in this figure represent the percentages of the normal outflow from the aorta obtained when the corresponding molar concentrations of adrenaline were perfused. It will be seen from the figure that the outflow falls to 12 per cent. of the normal when the molar concentration perfusing the vessel is 165×10^{-8} , but it will also be seen that this observation of 12 per/

per cent. lies quite above the general sweep of the curve. Reference has already been made in the previous paragraph to the difficulty met with in the attempt to determine what was 100 per cent. action, and the suggestion was offered that a complicating factor entered in to disturb the results when actions near the maximal were obtained, namely rapid weakening of the adrenaline solution. Since reduction of the outflow to 12 per cent. fell short of what was expected from the curve as maximal action, this observation was neglected. On the other hand, inspection of a curve similar to that in Fig. 4 but extended further along the abscissa suggested that 100 per cent. action would diminish the outflow to about 1.5 per cent. of the normal flow, and this was the value actually used as the basis for the subsequent calculations.

Fig. 4 /

Fig. 4.



We must now inquire whether the variations in the outflow which have been observed under adrenaline perfusions show concordance with Poiseuille's law for they are dependent on contraction of the circular muscle of the vessel wall, this contraction diminishing the circumference of the vessel, and with it of course the radius. Poiseuille's law shows how the rate of outflow from a tube of narrow calibre depends on a number of factors, among them being the radius/

radius of the tube. It is expressed in the formula -

$$A = \frac{(P_1 - P_2) \pi}{8 l \eta} \times r^4$$

where A = outflow from tube in unit time

P_1, P_2 = pressures at ends of tube

l = length of tube

η = coefficient of viscosity of fluid

r = radius of tube

In the experiments which have been described, all the factors on the right side of the equation were kept constant except r . The formula thus reduces to $A = kr^4$

$$= k \left(\frac{c}{2\pi} \right)^4$$

where c is the circumference of the tube.

i.e. $A = Kc^4$, for since 2π is a numerical term, $\left(\frac{1}{2\pi}\right)^4$ may be incorporated in the constant term of the formula.

Now, the circumference of the vessel is composed of plain muscle fibres the lengths of which vary under different concentrations of adrenaline according to the formula given in the previous section on the action of adrenaline on arterial strips, namely,

$$Kx = \frac{y}{100 - y}$$

It is therefore possible by utilising this formula and by taking the maximal action on the outflow as 1.5 per cent. of the normal outflow to construct the curve/

curve along which the observations should lie if Poiseuille's law does indeed apply as we have conjectured. This may be done as follows. From the observations made on the outflow changes, the lengths of circumference corresponding to each are calculated as percentages of the normal length according to Poiseuille's law. This has been done in Table II.

Table II.

Experiment 15, 24/2/27.			
(1) Molar Concentration of adrenaline $\times 10^{-8}$	(2) Variation in outflow observed expressed as per cent. of normal, i.e. A	(3) A (Variation in circum- ference) calculated from col- umn 2.	(4) Variation in circum- ference as per cent. of normal.
0.8	100	3.16	100
1.2	74	2.93	92.8
1.2	89	3.07	97.2
1.7	93	3.10	98.2
2.5	86	3.05	96.4
2.5	85	3.04	96.1
3.0	85	3.04	96.1
3.5	70	2.89	91.5
3.5	58	2.76	87.3
5.0	54	2.71	85.8
6.5	53	2.69	85.3
8.5	40	2.51	79.6
10.0	43	2.56	81.0
13.5	40	2.51	79.6
16.5	26	2.26	71.5
30.0	16	2.0	63.3
165.0	12	1.86	58.8

From this the curve relating circumferential length to concentration of adrenaline is drawn (Curve 2, Fig. 4). The length of the circumference when the action on the outflow is maximal (1.5 per cent. of the normal) is also calculated; it amounts to 35 per cent. of the normal length of the circumference. From this the length of the circumference which is 50 per cent. of the action on the plain muscle is obtained, namely 67.5 per cent. of the normal length. From Curve 2, Fig. 4, the molar concentration corresponding to this length is read off, 25×10^{-8} molar. Thus, in this experiment 50 per cent. of the action on plain muscle is obtained at the concentration 25×10^{-8} molar. The value of K in the formula $Kx = \frac{y}{100 - y}$ can now be calculated; it is 4×10^{-6} . The concentrations producing any percentage of the maximal action on the plain muscle can now be calculated. A suitable series of actions on the plain muscle is given in column 1 of Table III. The concentrations corresponding to these have been calculated in column 2 of the same table, while in column 3 the circumferential length corresponding to each action has been calculated as a percentage of the normal length from the datum that 100 per cent. action on plain muscle reduces its length to 35 per cent. of the normal. These percentages of the normal length given in column 3 are now/

now converted to fractions of 3.16 in column 4.

Column 4 was made simply for convenience; for we are trying to calculate the outflow which should result from a series of circumferential lengths knowing that the outflow varies as the fourth power of the circumference. 3.16 is the fourth root of 100, and if we express the circumferences as fractions of 3.16, then on raising these numbers to their fourth powers we obtain the outflows expressed as percentages of the normal. This has been done in column 5.

With regard to these calculations, it may at first sight appear that we have been arguing in a circle, for we have taken the fourth root of the observations of outflow variations to arrive at the circumferences corresponding, and then from circumferences we have returned, after a series of manipulations, by raising circumferences to their fourth powers, back to outflows. But actually this is not so. The object of taking the fourth roots of the observations on the outflow and then of drawing the curve relating the circumferences to the concentration was merely to obtain a pair of values for x and y in the equation $Kx = \frac{y}{100 - y}$ so as to evaluate K . The suitable value of K could theoretically have been arrived at without reference to the observations at all by the method of trial of different values, but of course the time spent in finding the proper value of K would have been enormous. Having arrived/

arrived at a suitable value of K we ignored the experimental findings except that for the maximal action on the outflow, viz. reduction to 1.5 per cent. of normal and built up the theoretical curve relating outflow changes to concentration by combining Poiseuille's law with the formula $4 \times 10^6 x = \frac{y}{100 - y}$

Table III.

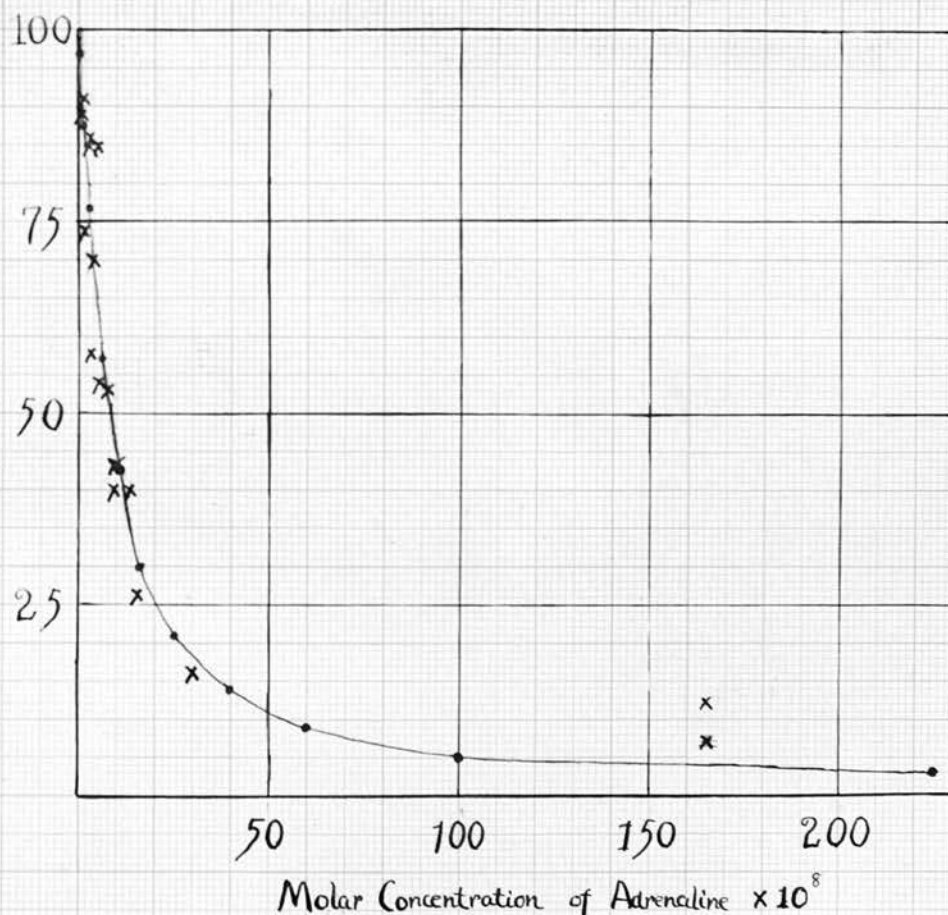
The figures in this table have been arrived at by the calculations described in the text.

Action on circumference expressed as per cent. of normal (y)	Molar concentration of adrenaline (x) $\times 10^6$	Variation in circumference expressed as percentage of normal length	Variation in circumference expressed as fraction of 3.16 ($\frac{1}{\sqrt{A}}$)	Variation in outflow (A)
0	0	100	3.16	100
1	0.25	99.35	3.14	97
5	1.05	96.75	3.06	87.6
10	2.8	93.5	2.96	76.7
20	6.5	87	2.75	57.1
30	10.7	80.5	2.55	42.25
40	16.2	74	2.34	30.03
50	25	67.5	2.14	20.88
60	40	61	1.93	13.84
70	60	54.5	1.73	8.95
80	100	48	1.52	5.34
90	225	41.5	1.32	3.03

In/

In Fig. 5 the circles lying on the continuous line represent the outflows listed in column 5 of Table III plotted against the corresponding concentrations listed in column 2 of the same table. This is the "calculated curve". The crosses show the outflows actually observed corresponding to particular concentrations; these are stated in columns 1 and 2 of Table II. The agreement between the observed and the calculated figures is satisfactory.

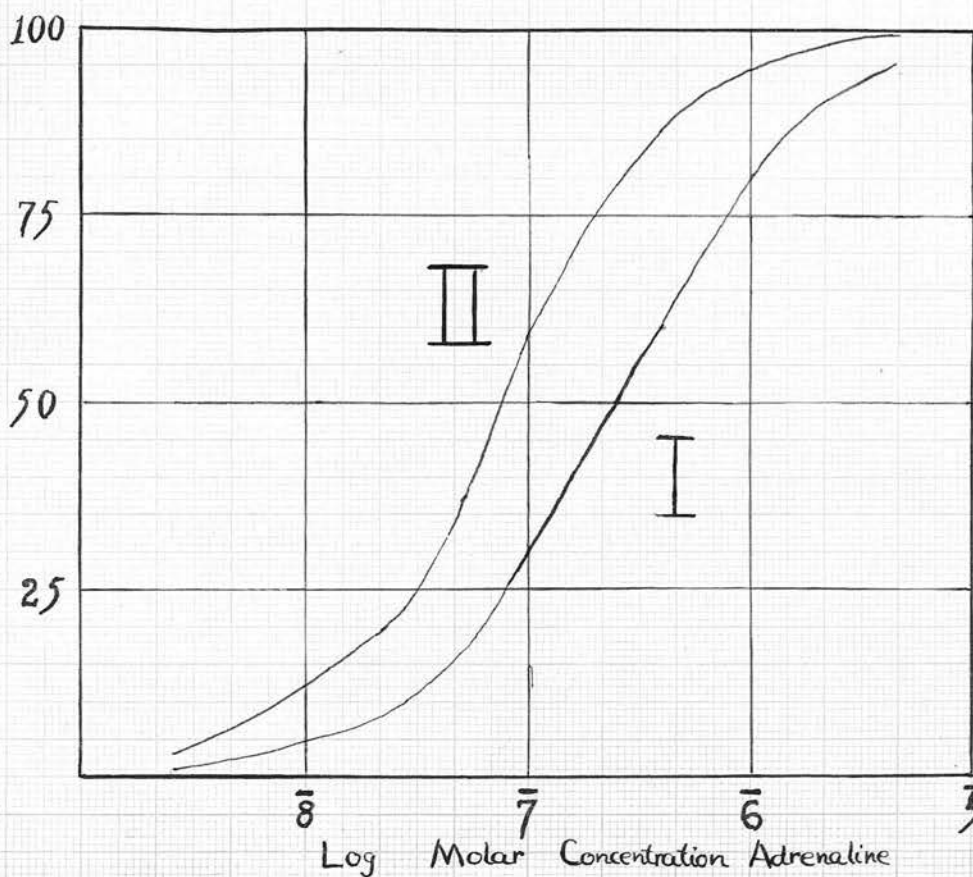
Fig. 5.



The series of experiments here recorded shows very clearly how profoundly ordinary physical laws modify the expression of the reaction which occurs between a tissue and a drug from the simple fundamental relation described in an earlier part of this paper. This is in part shown graphically in Fig. 6 . In this figure, curve I is the equation $Kx = \frac{y}{100 - y}$ which has been discussed when the strip experiments were described; in this case, K has the value 4×10^6 . The concentrations as before are plotted on a logarithmic scale and the curve has of course the same form as in Figs. 1, 2 and 3. In curve II, the ordinates represent the action of adrenaline on the outflow from the frog's aorta, these ordinates being taken from the figures calculated for the outflow in Table III, column 5. Both curves are theoretical. They show a considerable difference in form, curve II rising much more sharply. It will be noted that because of this sharper rise the chief effect of the modification produced by Poiseuille's law is to intensify the action of adrenaline at the lower concentrations especially. It must be admitted, however, that despite the introduction of Poiseuille's law the shapes of the curves are remarkably similar. But this shows only a part of the effect of Poiseuille's law. Far more important from the practical point of view is the widening of the limits within which the reaction may be obtained. This/

This is shown in Fig. 4 where it appears that the maximal action on the plain muscle shortens it to 35 per cent. of its normal length whereas the maximal action on the outflow reduces it to 1.5 per cent. of its normal rate.

Fig. 6.



These experiments may be termed isotonic, since the pressure of the perfusing fluid is kept at a constant level, namely, 40 cm. of water while changes dependent/

dependent on the variations in the circumference of the vessel are studied. They show a considerable difference from the isotonic experiments on the sheep's carotid, for the maximum action on the outflow in the experiment described in Table II was believed to occur when the length of the plain muscle fibres forming the circumference of the aorta was reduced to 35 per cent. of their initial size; whereas in the case of the sheep's carotid the maximal action occurred at about 70 per cent. of the initial length. The cause of the discrepancy in these results is probably to be found in the amount of non-contractile tissue present in the preparations; it is present in considerable amount in the sheep's carotid; while in the frog's aorta there is much less.

Up to this point we have studied the operation of only one of the factors in Poiseuille's law, namely, the effect of changes of radius (or circumference). Of the other factors in the equation expressing Poiseuille's law only one is of importance in the present study, the pressure factor. But this is a most important one for our present purpose, for it is a rise of blood pressure which is the most striking feature of the action of adrenaline in the intact animal, and it would seem desirable to study these alterations of pressure on the preparation which I have described. This could be done best by/

by adjusting the pressure with each different solution of adrenaline in such a way that the outflow from the preparation is constant. For this set of conditions, Poiseuille's law reduces to

$$A = (P_1 - P_2) \times r^4 \times \text{constant}$$

or, since A, the outflow, is constant,

$$(P_1 - P_2) \times r^4 = \text{constant.}$$

That is, the pressure should vary inversely as the fourth power of the radius (or circumference). It may be noted in passing that this type of experiment does not examine the isometric response of the plain muscle of the artery to adrenaline. Further, when the higher concentrations of adrenaline are used for perfusion, such as are expected to produce say an 80 per cent. action, the pressure would have to be raised to about twenty times its original amount if Poiseuille's law alone had to be reckoned with. This would necessitate applying the pressure by means of compressed air passing to the tube in the cork of the Mariotte flask; a manometer could be inserted into the side tube on the compressed air system to record the actual pressure developed. But in such experiments, where the pressure is the independent variable an important factor must enter to modify the operation of Poiseuille's law. For Poiseuille's law applies only to rigid tubes, whereas the frog's aorta is by no/

no means rigid. An elasticity factor must come into play so that the radius would vary in such experiments partly in accordance with the concentration of adrenaline, partly with the pressure. It would be necessary therefore to determine at the outset of each experiment how far the elasticity factor modified the operation of Poiseuille's law by determining the outflows at different pressures before any adrenaline was perfused. A considerable amount of calculation would then be called for to assign some value to the elasticity factor. We should expect such experiments to show that the effect of adrenaline on the vessel is partly to raise the tensions in the muscle cells, partly to diminish their lengths. Time, however, could not be found for experiments along these lines. The point is, however, important as we are now nearing the study of the blood pressure. It is illustrated by some figures published by Anrep and Daly⁽¹⁰⁾ which they obtained in the course of an investigation on the output of adrenaline in cerebral anaemia. Using the heart-lung preparation, they found that the heart perfusing the preparation was sensitive to high dilutions of adrenaline. Now, when adrenaline was injected into the aorta there was a rise of blood pressure and a diminution of the blood flow; no acceleration of the heart occurred, all the adrenaline being destroyed apparently before reaching the heart, so that the two effects were both wholly due/

due to the action on the vessels.

One result seems to follow from this. We cannot expect to determine the height to which the blood pressure should rise from the action of adrenaline, even on the vessels alone, by combining the formula for the action of adrenaline on plain muscle with Poiseuille's law, as we did to account for the changes in outflow under adrenaline. In Anrep and Daly's experiment for example, even if adrenaline solutions had been used to perfuse the animal instead of the injection of doses of adrenaline into the aorta, we should have eliminated the effect of adrenaline on the heart while maintaining a definite concentration of the drug in the vessels. But even so we should be unable to predict the height to which the blood pressure will rise, for the reasons that the effect of Poiseuille's law is exerted in two directions simultaneously, but in what proportion in each direction we do not know; and that the further factor of changes in the elasticity of the vessel walls must now come into play.

Action of Adrenaline on the Blood Pressure of the Cat.

The action of adrenaline on the blood vessels has been discussed in some detail. When we come to consider the action of adrenaline on the blood pressure, several complications are introduced. In the first place, the nervous system exercises a profound influence on the blood pressure. In the case of/

of adrenaline injection experiments the most prominent effect of the central nervous system is due to vagal action; it consists in a depression occurring in the blood pressure tracing during the rise of pressure following an injection of adrenaline. This deformation of the curves renders them useless for quantitative work since constancy of results cannot be attained. It is customary therefore to destroy part or whole of the central nervous system and to divide the vagi on both sides. Such experiments do not measure the effect of adrenaline on the blood pressure of the normal animal, and we are still far from being able to perform experiments of this kind, on animals in which the central nervous system is intact, with any hope of being able to explain the effects produced except in a very crude qualitative way. How far we are able to explain the effects produced when the central nervous system is eliminated as an interfering factor will appear later.

A second complicating factor is to be found in the manner in which the fluid is supplied to the vessels. In the experiments which were performed on the frog's aorta, the supply of fluid was kept constant from a reservoir. But when the blood pressure of an animal is studied, the fluid is contained in a closed system of vessels and heart which forms all the reservoir which there is; and the motive power is no longer the constant force of gravity but the heart beat. The heart beat itself is/

is subject to many influences. Among these is the inflow of fluid to the heart and this of course depends on the capacity of the vascular system; the greater that capacity, the less is the inflow and the heart beat is necessarily less effective. Now, capacity changes occur when the blood pressure rises from a vasoconstriction; we should expect the inflow of fluid into the heart to increase; actually, as Anrep and Daly's figures show, it is diminished. This is probably due to the increased aortic pressure causing a greater part of the energy of each heart beat to be expended before the aortic valves open. It now becomes obvious that the blood pressure depends upon a number of interdependent variable factors. Nevertheless it is convenient to group these factors on which the blood pressure depends in two large classes - those affecting the peripheral resistance and those affecting the output of the heart. Adrenaline has independent actions on the factors composing these two groups. The first group of factors consists of those which influence the peripheral resistance; the calibre of the vessels and the rate of flow of fluid through them are the main agents in the production of the peripheral resistance. The earlier part of this paper has been devoted to a study of the effect of adrenaline on these factors. The second group of factors includes the rate and strength of the heart beat and the filling/

filling of the heart. It is well known that adrenaline increases the rate and strength of the heart but its effect on these factors has not been studied separately here. Anrep and Daly, however, give figures showing the effect of adrenaline on the heart rate. When the increases in the heart rate are plotted against the corresponding concentrations a curve of hyperbolic form is obtained; but the range of concentration employed was only thirty-fold, so that the figures are insufficient to determine definitely whether the formula $Kx = \frac{y}{100 - y}$ can be applied to the action on the rate of the heart also.

The experiments were carried out in the following manner. A series of cats was first of all anaesthetised with ether, and then each animal was pithed by passing a wire rod right down to the end of the vertebral canal, artificial respiration being commenced as soon as this was done. An hour was allowed to elapse from the time of pithing before any injection of adrenaline was made; the object of this was to wash out the ether from the preparation as completely as possible before starting the injections, for it has been shown that the presence of ether depresses the vasomotor response to adrenaline (Wyman and Lutz⁽¹¹⁾). In the meantime the supra-renal veins were ligated on both sides. The blood pressure was recorded by a small tambour so as to prevent the entrance into the blood vessels of large/

large quantities of sulphate solution from the pressure bottle after large rises of blood pressure. Calibration of this tambour against a mercury manometer was done at the conclusion of each experiment.

A series of injections of adrenaline in various amounts was now given. The total bulk of fluid in each injection was 2 cc., the adrenaline solution being diluted with normal saline solution. At intervals 2 cc. of normal saline without any adrenaline were given to eliminate any errors due to the bulk of the injection. Each injection lasted for 15 seconds, the time being called out in 5 second intervals by an assistant who started a stop watch at the beginning of the injection. The importance of the duration of the injection was first pointed out by Reid Hunt⁽¹²⁾ who stated that the effect produced by a particular dose slowly given could be doubled by half that dose rapidly given.⁽¹³⁾ Cannon and Lyman also emphasise the effect which difference of rate of injection of the same dose produces upon the blood pressure responses. But before these differences in the blood pressure responses are obtained, the differences in the rate of injection have to be considerable, and the method of controlling the rate of injection adopted in the present series of experiments appears, though crude, to be quite accurate enough for the purpose.

In/

In one experiment the chest was opened and a cardiometer was fitted over the ventricles. It was hoped in this way to measure one of the two factors on which the blood pressure depends, namely, the cardiac output. The record showed that the first effect of the adrenaline injection was to increase the extent of cardiac contraction by causing more complete systole. Immediately after this the blood pressure rose sharply and with this the ventricle dilated rapidly and systole became much less complete than it was before the injection was given. The pressure next began to fall and now systole became much more complete, an extensive movement of the lever occurring at this stage; along with this the ventricle began to recover from its dilated condition, but much more gradually. From this it will be seen that it proved impossible to correlate the rise of pressure and the cardiac output changes in any quantitative way. For the two factors on which blood pressure depends influence one another: in this experiment the degree of cardiac dilatation with impaired systole which is present when the blood pressure is at its maximum shows how the rise of blood pressure consequent on increased peripheral resistance diminishes the output of the heart even when the rate and strength of the heart beat are stimulated as they are by adrenaline. The attempt to elucidate the action on the blood pressure by studying/

studying simultaneously with it one of the two interdependent factors on which it depends, namely the cardiac output, was therefore futile, and as no means exist of measuring the peripheral resistance along with cardiac output and blood pressure no further experiments were done along these lines.

The results of a typical blood pressure experiment are given in Table IV. In estimating the effect of adrenaline it was found better to take the difference between the systolic pressures before the injection and at the maximum after the injection rather than the absolute pressure to which the systolic pressure rose. Measurements of the diastolic pressures gave less constant results than the systolic measurements. Several injections of the same concentration were given and, as in the previous series of experiments high and low concentrations were alternated in irregular order.

At the beginning of an experiment, the usual level of systolic blood pressure was about 60 mm. of mercury. Thereafter it tended to fall; sometimes however it showed a long spread out wave so that after falling slowly for about an hour, a rise to about the original level occurred and then gradually the resting level subsided again. In no case was a fall of pressure ever observed after an injection of adrenaline. The absence of any depressor action of adrenaline in pithed cats is a well known difference/



difference in the responses of pithed and non-pithed cats to adrenaline. The work of Cannon and (13) Lyman suggests that the occurrence of the depressor action is conditioned by the presence of a certain degree of tonus in the vessel walls; in pithed cats the blood pressure is of course low and the tonus of the vessels is too low for the depressor action to appear.

Table IV /

Table IV.

11/3/27. Cat, male. 2830 grams.

Dose in mgms.	Serial no. of in- jection.	Systolic blood pressure in mm. of mercury		
		Before injection	After injection	Increase
.0002	15	56	64	8
.0005	14	57	75	18
.001	4	69	79	10
	5	64	75	11
	12	60	85	25
	16	61	84	23
.003	3	75	105	30
	10	46	78	32
	19	52	130	78
	20	46	130	84
.005	6	54	96	42
.01	2	77	158	81
	9	46	116	70
	17	60	172	112
	18	56	170	114
.02	13	51	190	139
.025	7	60	190	130
.03	11	46	182	136
.04	1	77	218	141
	8	62	204	142
.1	21	44	218	174
.25	22	48	232	184

The results of these experiments were disappointing. Constancy of response could not be obtained when the same amount of adrenaline was injected at long intervals, and the results show a wide divergence. The question of the constancy of the response to the same injection of adrenaline was originally investigated by Elliott⁽¹⁴⁾ and later by Jackson⁽¹⁵⁾. Elliott found that with the repetition of adrenaline the threshold of stimulation rises so that larger doses of the drug are required to produce the same effect. Jackson, however, found that in dogs approximately uniform responses may be obtained when the same dose is repeated many times, but eventually the response falls off. Elliott in a subsequent paper⁽¹⁶⁾ described a series of experiments upon cats in which he found that the blood pressure of cats responded to adrenaline "with the accuracy of a chemical balance". His method of preparation of the animal was to pass the pithing rod down the vertebral canal to the fourth dorsal vertebra. Table IV shows, however, marked differences in the responses to the same dose; but while this is so these marked differences occur only when the doses were widely separated in time. When the doses were consecutive the responses are remarkably close in value. In fact up to the tenth or eleventh injection the responses may be said to be uniform; thereafter the preparation seems to become more sensitive to adrenaline/

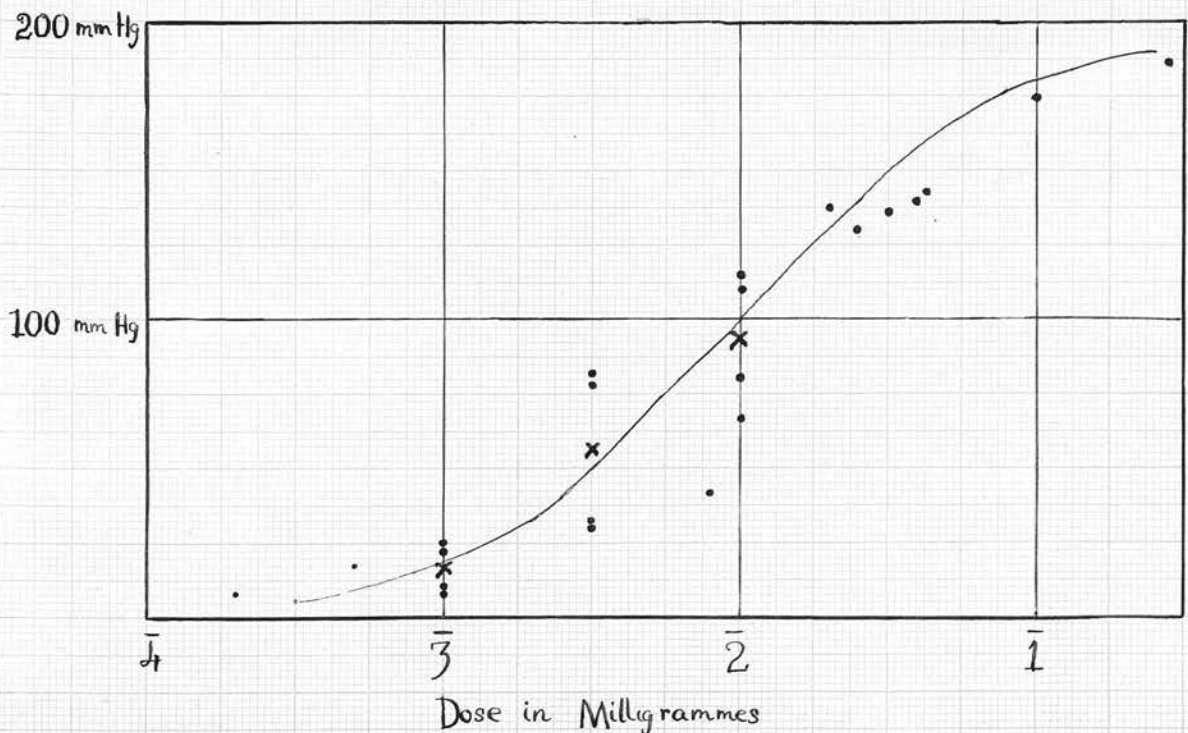
adrenaline and larger effects are produced by the same dose.

This augmentation of the blood pressure response to adrenaline has been noted by many workers (Levy, Lieb and Hyman, and others). It seems that (17) the work of Lutz and Wyman may throw light on this question. These workers found that in most cats anaesthetised in the ordinary way with ether and in pithed cats the blood pressure responses to adrenaline after a certain interval from the beginning of the experiment showed a progressive rise in the sensitivity of the preparation to the drug. The usual interval after which augmentation of the response appears is given as two hours from the time of pithing, while the maximum effect was attained in three hours. The amount of augmentation was usually about 100 per cent. but it varied between such wide limits as 30 and 600 per cent. They state that these phenomena are not dependent on the amount of pithing nor on the level of the blood pressure: they are in fact unavoidable. They correlate these vascular phenomena with changes in the CO_2 capacity of the blood. These investigations explain very well the divergences in Table IV in the results following the injections of equal doses of adrenaline.

The question now arises as to what value can be attached to the results given in Table IV since they are subject to such wide variations. In Fig. 7, the/

the average of the responses produced by the same dose has been taken (crosses); in this way the change in sensitivity of the preparations is minimised. Less reliance can be placed on the results given for doses which were tried only once. It must be admitted, however, that the method of averaging the results is very crude. The individual observations are shown by small circles in the figure. The range of dosage employed necessitated its being plotted on a logarithmic scale. The dose effect curve shows a practically linear relationship between the effect and the logarithm of the dose over a wide range of concentration, but at the lower concentrations this linear relationship ceases to apply and the graph descends less steeply as it approaches the zero level of effect.

Fig. 7.



The relation of the dosage of adrenaline to its action on the blood pressure has already been studied (12) by Reid Hunt . Reid Hunt's experiments are very briefly reported and his findings may be summed up by the statement that the blood pressure responses do not bear a linear relationship to the dosage. The (18) question was later re-investigated by Murray Lyon . This investigator found that the Weber Fechner law was applicable to the response of the blood pressure to adrenaline, and results were given which showed that the observed figures were fitted by the formula

$$E = N \log_e S + C$$

where E = effect on blood pressure

N = constant (expressing sensitiveness of the reacting mechanism)

S = amount of adrenaline

C = constant

The results given in Table IV confirm this finding over the range of concentration used; but they also show that results can be obtained over a much wider range of concentration than Murray Lyon employed and that these results show a considerable divergence from the simple relation which he suggests.

The blood pressure experiments are rather unsatisfactory. It is impossible to obtain the necessary constancy of results required to establish any formula as an adequate description of the actual relation between the blood pressure and the dosage.

This/

This is not surprising when it is kept in mind how large a number of factors are concerned in the production of the blood pressure. The question is rendered the more complicated by the fact that some of these factors are apparently chemical if the views of Lutz and Wyman are correct.

Several investigators have studied the relationship between the dosage of various drugs and the effects produced. It has been necessary to refer to a few of these studies already in earlier sections of this paper in order to explain the experiments which were performed and their results. But now a few comments may be offered on some of the ideas contained in these studies.

Attempts have been made to bring all sorts of reactions, reversible and irreversible, simple and complicated within the scope of a single law or formula. Shackell^(7, 19) for example attempts to bring the responses of arterial rings to adrenaline and the toxic effects of a series of antiseptics both within the scope of the Law of Probability. It has already been urged on experimental grounds that such a view is incorrect, and no further discussion of the point will be undertaken now. But it is interesting to note that Shackell assumes that because he has obtained S-shaped concentration-action curves from his studies of the action of antiseptics on/

on lowly marine organisms and also from his experiments with adrenaline on arterial rings that similarly shaped curves should be obtained when the action of adrenaline on the blood pressure is examined.

A similar idea is contained in a paper by Cameron and Mackersie (20). This paper summarises the results supplied in a number of studies on the subject. These studies included the most diverse reactions - the effect of insulin on the blood sugar, of pituitrin on plain muscle, Shackell's study of the action of adrenaline on arterial rings, the toxicity of the nitrate anion on frog's muscle, Cash's study of the effect of indaconitine on temperature, the effect of vitamin B on growth, Murray Lyon's study of the effect of adrenaline on the blood pressure. From these various studies they conclude that all cases are covered by the formula $y = \log (x + 1)$, where y = effect and x = dose of drug. The authors themselves state that their equation can be but a first approximation since it utterly fails to fit actions approaching the maximal. It should be realised as well however that the data from which these authors worked were drawn from experiments on reactions whose nature was not understood. It is not known how insulin does lower the blood sugar; it is not known how vitamin B influences growth, nor is it known how parathyroid extract raises the blood calcium. These reactions may be simple or they may/

may be highly complicated; they may comprise a whole series of chemical reactions. Such reactions are in fact too crude and little understood to be used for the elucidation of reactions occurring between the drug and the cell.

Similarly the action of adrenaline on the blood pressure. It has been shown in this paper how large a number of variable factors come into play when a dose of adrenaline raises the blood pressure. In the first place the relation between the concentration of the drug and the amount of contraction of the plain muscle cells was examined; it was found that the relation was described by the formula for a reversible reaction. The next step was to consider what factors could conceivably modify this formula as a description of the action of adrenaline on the blood pressure. The most obvious factor was Poiseuille's law which describes the flow of fluids through tubes; it was found that this law did apply when alterations in radius of the artery were produced by adrenaline, and it was inferred that Poiseuille's law must play a part in modifying any attempt to apply the formula mentioned to explain the changes in the peripheral resistance to the blood flow. The probable importance of the elasticity of the arteries in the production of the peripheral resistance was pointed out. The problem was next considered from the side of the cardiac output; this was very briefly discussed and no experiments were done on this point. Finally/

Finally the effect of intravenous injections of adrenaline was examined. Unfortunately the results were unsatisfactory, but such as they were they showed a remarkably close agreement with the formula $Kx = \frac{y}{100 - y}$ (The continuous line in Fig. 7 is this equation). We have already seen (when the outflow changes from the frog's aorta were studied) that even when the fundamental formula is overlaid by some physical law the concentration action curves retain a very similar shape (see Fig. 6). It may be that the same explanation holds here. It is of course incredible that the simple unimolecular formula $Kx = \frac{y}{100 - y}$ alone is sufficient to explain the blood pressure changes under adrenaline. It must be combined with several other relations introduced by the structure of the circulatory system before the true relation connecting the blood pressure response with the dose can be arrived at. The true relation is therefore bound to depart from the simple formula given in several particulars although perhaps retaining a general similarity of contour to it.

Now there is no reason to suppose that all drugs follow the same formula as adrenaline in their interactions with the cells of the tissue. Straub's work suggests that the contrary is the case. Moreover the physical conditions introduced into the reaction between a drug and some function of the body /

body vary with the system which is studied. It follows that it is unsound to refer all sorts of reactions to a single formula in the wholesale fashion in which Cameron and Mackersie have done this. As Cash remarks: "It is improbable that any universal law of uniform applicability associating dosage with its certain effect could be formulated which would prove adaptable to all classes of remedies."

Conclusions

1. The reaction between adrenaline and the plain muscle cells of the arteries proceeds according to the formula
$$Kx = \frac{y}{100 - y}$$

when x = molar concentration of adrenaline

y = action produced expressed as percentage of maximal action

K = constant.

This indicates that a reversible unimolecular reaction occurs between the drug and some receptors in the cell.

2. Certain physical laws, introduced by the structure of the circulatory system interfere with the direct applicability of this formula to the blood pressure changes produced by adrenaline. Poiseuille's law has been shown to account for the difference between the variations in the outflow of fluid/

fluid from an artery and the variations in length of an arterial strip produced by adrenaline.

3. When the intravascular pressure changes under adrenaline are considered, the applicability of Poiseuille's law to these phenomena is shown to be itself modified by changes in the rate of flow and by the elasticity of the vessel walls.

4. The responses of the blood pressure to intravenous injections of adrenaline show that a linear relationship exists between the effect produced and the logarithm of the dose over a considerable range of concentration; but for the lowest effective concentrations of adrenaline this relation does not hold and the effect tends to vary as the dose.

Viewing the results as a whole there is a surprisingly close agreement between them and the formula

$Kx = \frac{y}{100 - y}$ given above for the reaction between the muscle cells and the drug.

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